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# Local outbreak of *Listeria monocytogenes* serotype 4b sequence type 6 due to contaminated meat pâté

Denise Althaus [a], Marco Jermini [b], Petra Giannini [b], Gladys Martinetti [c], Danuta Reinholz [d], Magdalena Nüesch-Inderbinen [a], Angelika Lehner [a], Roger Stephan [a, *]

[a] Institute for Food Safety and Hygiene, National Centre for Enteropathogenic Bacteria and Listeria, Vetsuisse Faculty University of Zurich, Zurich, Switzerland

[b] Cantonal Laboratory Ticino, Division of Public Health, Department of Health, Bellinzona, Switzerland

[c] Laboratory of Bacteriology, Department of Laboratory Medicine, Ente Ospedaliero Cantonale, Bellinzona, Switzerland

[d] Office of the Chief Medical Officer of Canton Ticino, Division of Public Health, Department of Health, Bellinzona, Switzerland

**Running title**: L. monocytogenes 4b ST6, pâté, outbreak

*Corresponding author

Roger Stephan, Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Winterthurerstrasse 272, CH-8057 Zurich, Switzerland.

Phone 0041-44-6358651, Fax 0041-44-6358908, e-mail stephanr@fsafety.uzh.ch

**Abstract**

In January and February 2016, five cases of confirmed and two cases of probable infection due to *Listeria monocytogenes* serotype 4b, sequence type 6 belonging to a single PFGE pulsotype pattern were registered in a region of southern Switzerland. *L. monocytogenes* was detected in blood samples (four cases), and pleural fluid (one case). Furthermore, *L. monocytogenes* 4bST6 was detected in a stool sample of an asymptomatic person exposed to a common food. Forthwith, the food safety authority and a local gourmet meat producer reported *L. monocytogenes* contamination of meat pâté. Analysis of further food and environmental samples from the premises of the producer yielded isolates matching the clinical strains and confirmed the presence of *L. monocytogenes* 4bST6 in the mincing machine as the cause of the food contamination.

**Key words**

outbreak, *Listeria monocytogenes*, sequence type 6, pâté.

## Introduction

*Listeria monocytogenes* is a foodborne pathogen with the ability to multiply at refrigeration temperatures, and to persist in food-processing environments and equipment. Listeriosis, although rare, is a potentially fatal infection, with the growing number of the elderly in the population, pregnant women and immunocompromised persons at particular risk (Allerberger et al., 2015). In 2014, the EU notification rate was 0.52 cases per 100,000 population, representing a 30% increase compared with 2013 and reflecting a statistically significant increasing trend of listeriosis between 2008-2014 (EFSA, 2015). Although most cases of listeriosis are sporadic, small and large outbreaks occur regularly (Hernandez-Milian & Payeras-Cifre, 2014). So far, four outbreaks of listeriosis have been reported in Switzerland involving soft cheese in two outbreaks, cooked ham and ready-to-eat salad in one outbreak each, respectively (Büla, Bille, & Glauser, 1995; Bille et al., 2005; Hächler et al., 2013; Stephan et al., 2015).

We describe a local outbreak of listeriosis that occurred in the canton of Ticino, located in southern Switzerland, during the Christmas holiday season of 2015/2016 due to contaminated meat pâté. The pâté was sold or distributed as a component of uncooled gourmet gift baskets. The first indication of an outbreak was obtained from the hospital laboratory services and the regional food safety authority by means of the Swiss mandatory reporting system for infectious diseases.

## Materials and Methods

*Clinical data of the patients*

Anonymised anamnestic data (data derived from the patients by the physicians) were provided, under the supervision of the office of the Chief Medical Officer, by the hospital attending to the patients. For each patient, data included age, gender, date of onset of symptoms and date of positive blood cultures and, where available, date of receipt and consumption of the pâté. The case definition for listeriosis used in this study is in accordance with the case definition by the European Commission (EC, 2012). Thereby, five patients were confirmed cases (with isolation of *L. monocytogenes* from normally sterile sites), two patients were probable cases (meeting clinical criteria and with exposure to a common food) and one case was an asymptomatic fecal carrier with exposure to a common food). Laboratory confirmed cases of *L. monocytogenes* infection were reported to the Swiss National Notification System for Infectious Diseases (NNSID) of the Swiss Federal Office of Public Health (SFOPH).

*Outbreak detection and microbiological analysis*

With the first suspicion of invasive *L. monocytogenes* infection due to consumption of pâté, food and environmental samples from the producing company, a local gourmet meat factory, were analysed. Quantitative detection of *L. monocytogenes* was done using the mandatory method for official laboratories of food control based on the International Organization for Standardization (ISO) ISO 11290-2. Qualitative detection of *L. monocytogenes* was performed using an accredited rapid method (AFNOR: AES 10/3 – 09/00). Eighteen samples obtained from a total of 4,500g of impounded pâté were investigated for the growth of *L. monocytogenes* over period of 12 days (t0-t12), which represented the company-declared

shelf-life of the pâté. Samples yielding ≥10 colony forming units (CFU)/g of pâté at t0 were analysed at time points t3, t6, t10 and t12. All other samples were analysed at t0 and t12. Storage temperature was set at 9°C, to simulate temperature abuse by consumers. Isolates were sent to the National Centre for Enteropathogenic Bacteria and Listeria (NENT) for final confirmation and further characterisation.

*Serotyping*

Serotyping of *L. monocytogenes* strains was performed using the commercial set of Listeria O-factor and H-factor antisera from Denka Seiken (Pharma Consulting, Burgdorf, Switzerland).

*Pulsed-field gel electrophoresis*

PFGE analysis of the strains was done as described by Althaus et al. (2014) in accordance with the CDC PulseNet protocol (http://www.cdc.gov/pulsenet/pathogens/ index.html).

*Multilocus sequence typing*

Multilocus sequence typing (MLST) was performed as described by Ragon et al. (2008). Sequences were imported into the Institut Pasteur MLST database website (www.pasteur.fr/mlst) to determine sequence types (ST).

## Results

*Recognition of the local outbreak*

*L. monocytogenes* was isolated between January 4th and January 8th 2016, from blood samples of two patients presenting to a local hospital in Ticino, Switzerland, over the Christmas holiday season of 2015/2016 with symptoms of systemic infection. Assessment of anamnestic data by the treating physicians and clinical microbiologists pointed to the ingestion of a common food, a locally produced meat speciality, which the patients reported eating. Subsequently, the local food authority was involved and *L. monocytogenes* (isolate N16-0072, Table 1) was isolated from a sample of pâté suspected of contamination. Serotyping and multilocus sequence typing identified all isolates as serotype 4b ST6. The clinical isolates and the food isolate were indistinguishable in their PFGE patterns and distinct from other clinical isolates of *L. monocytogenes* 4b analysed during the same period from the same region.

*Clinical data of the outbreak patients*

In total, seven (five confirmed and two probable) outbreak-related cases were registered. Thereof, six had presented to the emergency department of local hospitals with symptoms of food poisoning. The first confirmed case had consumed pâté on December 19th, 2015, at a large pre-Christmas gathering, and the second in early January 2016 at a family meal at home. Two further confirmed cases and two probable cases belonged to a group of six people who had eaten pâté together at a lunch gathering just before Christmas 2015. Onset of symptoms occurred between one and eight days after consumption (Table 1). Symptoms included fever (50%), diarrhoea (37.5%), vomiting (20%), chills, (20%) and neurological impairment (20%). Results of blood cultures were positive in four cases (Table 1). For one

asymptomatic person belonging to the group of six people, results of stool culture were positive for *L. monocytogenes* serotype 4b ST6 (isolate N16-0136, Table 1). A further case was registered four weeks later at the same hospital. The patient, who presented with fever, had confirmed growth of *L. monocytogenes* serotype 4b ST6 isolated from pleural fluid (isolate N16-0348, Table 1). The patient reported having eaten pâté at an unspecified date around Christmas 2015. Incubation time was estimated at >50 days. The median age of the patients with confirmed listeriosis was 84.8 years (range 71-95) and 71.4% were male (Table 1).

*Qualitative investigation*

The majority of the patients reported purchasing or receiving the pâté as component of a gourmet gift package issued by a local gourmet meat producing company shortly before Christmas 2015. The pâté, produced and frozen as a batch of approx. 260 kg on November 21$^{st}$, 2015, had been thawed and re-portioned for distribution in the week between the 16$^{th}$ and 23$^{rd}$ of December 2015. Distribution had occurred with intermittent lapses in temperature control. After the first pâté sample was culture-positive for *L. monocytogenes* (isolate N16-0072), the product was withdrawn from the market. Simultaneously, the production company reported to the regional food safety authority the detection of *L. monocytogenes* from four samples of pâté (isolates N16-0123 to N16-0126, Table 1). Based on these results, further environmental samples were officially obtained from the premises of the company. The meat mincing machine was completely disassembled and all cutter blades and plastic gear parts were sampled with environmental swabs. Since visual evidence indicated that the machine was highly suspect, no other locations on the premises were sampled. All samples from the machine resulted positive for *L. monocytogenes* (Table 1). The isolates were indistinguishable in their PFGE patterns from the clinical and the food isolates.

*Enumeration and growth of L. monocytogenes serotype in pâté samples*

Growth of *L. monocytogenes* was detected in all 18 samples obtained from the impounded pâté ($a_{w:}$ 0.967, pH: 6). Initial values of ≥10 CFU/g were observed for three (16.6%) of the samples. At the end of shelf-life, 16 (88.9%) of the samples exhibited >100 CFU/g of *L. monocytogenes* with a median of $1.3x10^6$ CFU/g (range $6.4x10^3$ –$7.5x10^6$).

**Discussion**

In this outbreak, the early raising of suspicion by the medical staff and the food safety authority allowed rapid recognition of the source and re-establishment of food safety for consumers. Indeed, in Canton Ticino, because of a higher incidence of listeriosis (3.43 cases per 100,000 population in 2014 and 1.4 cases per 100,000 population in 2015) than elsewhere in the country, the food safety authority and the chief medical officer agreed that each patient with suspected listeriosis should be interviewed about food consumption during the 4 weeks before symptoms onset. Swiss as well as EU food legislation decrees that *L. monocytogenes* must be absent from 25 g of food in which it can grow, unless the producer can demonstrate that it will not exceed the limit of 100 CFU/g throughout its shelf-life (EFSA, 2015). In this case, the producer did not have any shelf-life validation data and the food safety authority was able to demonstrate growth and presence of the pathogen above this limit at expiration date. Furthermore, uncontrolled temperature along the distribution process of the product may have contributed to high *L. monocytogenes* colony counts at consumer level. Pheno- and genotyping of the isolates could link the contamination to specific parts of a food processing machine on the premises of the producer. Serotyping assigned the strains to 4b which is the second most frequently isolated serotype in various food matrices as well as human listeriosis cases in Switzerland (Althaus et al., 2014; Ebner et al., 2015). Further, the strain associated

with the outbreak described in this study belonged to ST 6. This is not the same ST detected in previous outbreaks in Switzerland. ST6 accounted for 4/93 (4.3%) cases of human listeriosis in Switzerland between 2011 and 2013 (Althaus et al., 2014). Although rare, this ST is associated with significantly worse outcome in cases with listeria meningitis (Koopmans et al., 2013) and therefore poses a particular threat to consumer health. Cases of listerial pleural fluid as described in one of the outbreak patients are very rarely reported in the literature. In contrast to other pleural infections, they are caused by transient bacteraemia, rather than pulmonary infection (Mazzulli & Salit, 1991). To our knowledge, this is the only report clearly linking pleural infection with listeria to the ingestion of contaminated food.

## Conclusions

In the case of food-borne outbreaks, the main objective is the rapid identification and suppression of the infectious source. Source identification, allowing the authorities to re-establish food safety for consumers often involves a combination of epidemiological and microbiological techniques. Pheno- and genotyping of isolates from clinical cases, products and the production environment enabled in this outbreak situation the identification of the spreading source for this *L. monocytogenes* strain. To prevent similar outbreaks in future, and to protect consumers from exposure to listeriae, it is needful to stress the importance of good hygiene practices (including careful cleaning and sanitation of all parts of equipment), effective temperature control throughout the food production and distribution, as well as careful setting and validating of appropriate shelf life.

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## Disclosure Statement

No competing interests exist.

## References


Allerberger F, Bagó Z, Huhulescu S, Pietzka A. Listeriosis: The dark side of refrigeration and ensiling. In: *Zoonoses - infections affecting humans and animals: Focus on public health aspects*. Sing A, (ed.). Heidelberg, Germany: Springer Verlag, 2015, pp. 249-286.

Althaus D, Lehner A, Brisse S, Maury M, Tasara T, Stephan R. Characterization of Listeria monocytogenes strains isolated during 2011-2013 from human infections in Switzerland. Foodborne Pathog Dis 2014;11:753-758.

Bille J, Blanc DS, Schmid H, Boubaker K, Baumgartner A, Siegrist HH, Tritten ML, Lienhard R, Berner D, Anderau R, Treboux M, Ducommun JM, Malinverni R, Genné D, Erard Ph, Waespi U. Outbreak of human listeriosis associated with tomme cheese in northwest Switzerland, 2005. Euro Surveill 2005;11:91-93.

Büla CJ, Bille J, Glauser MP. An epidemic of food-borne listeriosis in western Switzerland: description of 57 cases involving adults. Clin Infect Dis 1995;20:66-72.

Ebner R, Stephan R, Althaus D, Brisse S, Maury M, Tasara T. Phenotypic and genotypic characteristics of *Listeria monocytogenes* strains isolated during 2011-2014 from different food matrices in Switzerland. Food Control 2015;57:321-326.

EC (European Commission). Commission decision of 28 April 2008 amending Decision

2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council. O J (L262) 2012;27.9:19-20.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA Journal 2015;13:4329, 191 pp. doi:10.2903/j.efsa.2015.4329.

Hächler H, Marti G, Giannini P, Lehner A, Jost M, Beck J, Weiss F, Bally B, Jermini M, Stephan R, Baumgartner A. Outbreak of listeriosis due to imported cooked ham, Switzerland 2011. Euro Surveill 2013;18:20469.

Hernandez-Milian A, Payeras-Cifre A. What is new in listeriosis? Biomed Res Int 2014; Article ID 358051: 7pp. doi: 10.1155/2014/358051.

Koopmans MM, Brouwer MC, Bijlsma MW, Bovenkerk S, Keijzers W, van der Ende A, van de Beek D. *Listeria monocytogenes* sequence type 6 and increased rate of unfavorable outcome in meningitis: epidemiologic cohort study. Clin Inf Dis 2013;57:247–253.

Mazzulli T, Salit IE. Pleural fluid infection caused by *Listeria monocytogenes:* case report and review. Rev Infect Dis 1991;13:564-70.

Ragon M, Wirth T, Hollandt F, Lavenir R, Lecuit M, Le Monnier A, Brisse S. A new perspective on *Listeria monocytogenes* evolution. PLoS Pathog 2008;4: e1000146.

Stephan R, Althaus D, Kiefer S, Lehner A, Hatz C, Schmutz C, Jost M, Gerber N, Baumgartner A, Hächler H, Mäusezahl-Feuz M. Foodborne transmission of *Listeria monocytogenes* via ready-to-eat salad: a nationwide outbreak in Switzerland, 2013-2014. Food Control 2015;57:14-17.